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Title of the Invention

A FOUR DIMENSIONAL BIOCHIP DESIGN FOR HIGH THROUGHPUT APPLICATIONS AND METHODS OF USING THE FOUR DIMENSIONAL BIOCHIP

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a 4D biochip, methods for its use and methods for its production, wherein the 4D biochip provides high efficiency and high throughput testing.

Discussion of the Background

Large scale, multiple sample, parallel biochemistry assays, automated instruments, and system integration (instrument, databases and analytical tools) using the latest bioinformatics technologies are key factors for advancing the field of functional genomics. In recent years, DNA chip technology has been a focal point of genomic scientists and potential customers of genomics technology because of the ability of the DNA chip to assay a large number of genes in parallel. DNA chip technology can be used, for example, in gene expression assaying (parallel Northern blotting) to determine gene functions, in polymorphism detection and molecular marker genotyping (for example, SNP), to provide efficient genetic mapping, and, most importantly, in human disease diagnostics and in phenotype prediction for genetic manipulation of plants and animals. Further, the integration of DNA chip and protein characterization data is an important step in correlating the results of genomic and protemic studies.

There exist several DNA chips which are used to conduct multiple sample parallel bioassays. For example, U.S. Patent Nos. 5,800,992 and 5,744,305 to Fodor et al. discloses an oligonucleotide-based chip (herein called the "Fodor chip") and U.S. Patent No. 5,807,522 to Brown et al. discloses a cDNA-based microarray chip (herein called the "Brown chip"). Conventional biochips (the Fodor and Brown chips, and other two dimensional or surface-based biochips such as the Affymetrix type of biochip and the Stanford type of glass-slide microarray or Bio-Informatics Group's 3D biochip) are designed for parallel assays of a large number of bioelements (e.g. genes) for a single biosample (e.g. patients) or parallel assays of a single gene for a number of biosamples. For conventional biochips, data for different biosamples are usually generated at different times or under different experimental conditions. Experimental errors are the most common obstacles for the usefulness of the experiment because they prevent accurate comparison between the biosamples, which usually is the goal of the experiment.

The Fodor chip (U.S. Patent Nos. 5,800,992 and 5,744,305) generally utilizes a flat silicon surface for *in situ* synthesis of the oligonucleotides on the chip surface using combinatory chemistry. The Fodor chip is typically limited to short oligonucleotide lengths, where the oligos have a small number (ie: 25) of nucleotide bases. The Fodor chip, therefore, may also be limited by experimental error associated with on-chip oligonucleotide synthesis and with short oligonucleotide hybridization error, which is generally associated with non-specific hybridization in a relaxed condition. Thus, due to these inherent experimental errors, techniques utilizing the Fodor chip may be prone to poor experimental repeatability. In addition, the Fodor chip may further be limited by slow hybridization rates due to the small effective hybridization area and random probe solution flow on the chip surface. In

some instances, RNA amplification may also be required to increase the RNA concentration in the probe solution, which may make the Fodor chip unsuitable for certain applications. Thus, procedures involving the Fodor chip may be cost inefficient due to the complexities and limitations involved in producing the chip (labor intensive and time consuming), capturing the necessary images, and analyzing the collected data.

The Brown chip (U.S. Patent No. 5,807,522) utilizes cDNA samples disposed in a microarray on the surface of a chip comprising a glass slide. The cDNA segments are typically chosen from cDNA libraries of EST sequencing projects. Each cDNA segment may range in length from several hundred to several thousand nucleotides. The nucleotide sequences in the cDNA segments are generally known, though cDNA segments without nucleotide sequence information and synthetic oligos may also be used in fabricating a Brown chip. The cDNA samples are usually delivered onto the chip using a robot having a three-dimensional motion control system and the ability to concurrently deposit multiple samples using a plurality of spotting pins. However, the Brown chip may also experience limitations such as, for example, error in the x-y positioning of the spotting pins by the robot and varying amounts of the cDNA samples deposited at each spot on the chip. In addition, hybridization error may be a limiting factor due to the small effective hybridization area on the chip and possibly due to secondary structure formed by single-strand oligonucleotides. Further, techniques using the Brown chip may be subject to extended hybridization times measured, for instance, in hours (for example, overnight hybridization). As with the Fodor chip, the Brown chip may also be difficult to produce and may be limited in its practicality due to the limited surface area available on the chip.

Thus, the DNA samples reside on the surface of, for example, a glass slide or a silicon wafer according to both the Fodor and the Brown DNA chips. However, though the Fodor and Brown DNA chips are useful for some small-scale research in functional genomics, they are not suitable for future practical applications primarily due to high cost, time intensive fabrication of the DNA chip, and poor accuracy of experimental results. The poor accuracy of a surface-based biosample assay apparatus and method, for example, according to both Fodor and Brown, typically results from the low concentration of the complementary strands of DNA (or RNA) in the probe solution and the small effective hybridization area of the spots on surface-based chips. The biosamples are usually introduced to conventional biochips by hand or a robotic liquid handling instrument which is time consuming and subject to mistakes in the liquid handling. Further, the surface-based chips, such as the Brown chip, are often prepared using the robotic system for transferring biosamples from a mass solution to individual spots on a glass substrate to form the microarray. The biosample transfer may be accomplished, for example, by a robot operating at an overall rate of about four dots per second. Since a microarray may include, for instance, multiple thousands of individual samples, a microarray may be prone to lengthy formation times as well as possible contamination due to the robotic system.

The limited surface area and between sample variance for these conventional biochips points out a need in the art for a biochip based technology that can drastically increase the number of bioelements, the number of biosamples, or both being tested, and minimize between sample variances by allowing simultaneous testing of large numbers of biosamples.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a perspective view of a 3D biochip.

Figure 2 is a perspective view of a capillary defined in a 3D biochip.

Figure 3 is a cross-sectional view of a 3D biochip.

Figure 4 is a perspective view of a 4D biochip or cube.

Figure 5 is a perspective view of a volume-reducing arrayer for depositing a predetermined amount of a liquid on a substrate according to one embodiment of the present invention.

Figure 6 is a side elevation of a volume-reducing arrayer for depositing a predetermined amount of a liquid on a substrate according to an alternate embodiment of the present invention.

Figure 7A is a schematic of a volume-reducing liquid arrayer for depositing a predetermined amount of a liquid on a substrate according to one embodiment of the present invention.

Figure 7B shows top and bottom views of each plate comprising a volume-reducing arrayer according to the embodiment of the present invention shown in FIG. 3A.

Figure 8A is a schematic of a volume-reducing arrayer for depositing a predetermined amount of a liquid on a substrate according to an alternate embodiment of the present invention.

Figure 8B shows top and bottom views of the plates comprising a volume-reducing arrayer according to the embodiment of the present invention shown in FIG. 4A.

Figure 9 is a perspective view of a volume-reducing arrayer positioned on a 4D biochip.

Figure 10 is a perspective view of the delivery of probes onto a 3D biochip/4D biochip with parallel tubing.

Figure 11 is a perspective view of delivery of reagents, probes and/or test samples with a syringe.

Figure 12 is a perspective view the positions of overflow tubing in a biochip delivery apparatus.

SUMMARY OF THE INVENTION

Accordingly, one object of the present invention is to provide a 4D biochip assembly that provides increased efficiency and throughput relative to conventional biochips.

A further object of the present invention is to provide a 4D biochip that can increase sample throughput by up to several orders of magnitude relative to conventional biochips.

A further object of the present invention is to provide a method for testing or assaying large numbers of samples simultaneously against the same bioelement or bioelements with high quality control.

A further object of the present invention is to provide a method for testing or assaying a sample or samples against large numbers of bioelements simultaneously.

A further object of the present invention is to provide a method for the rapid and efficient preparation of a 4D biochip of the present invention.

These and other objects of the present invention have been satisfied by the discovery and development of an article comprising:

at least two plates, wherein each plate defines a plurality of cylindrical capillaries, each capillary having a pair of opposed ends, with at least one capillary comprising a reagent inlet and at least one capillary comprising a reagent outlet;

the plate further defining a plurality of channels oriented substantially perpendicularly to the capillaries and configured to selectively operably connect adjacent capillaries so as to form a continuous passage from the reagent inlet to the reagent outlet, the channels being further configured to direct the reagent into a capillary at one end thereof and from the capillary at the other end thereof such that the reagent flows through substantially the length of the capillary and serially through all of the capillaries defined by the plate; and

wherein each of the at least two plates are positioned to substantially align the plurality of cylindrical capillaries between each pair of adjacent plates, and the use of this apparatus in high throughput, high efficiency screening methods.

In a further embodiment, the objects have been satisfied by the discovery and development of a 4D biochip, comprising:

m 3D biochip means, wherein *m* is an integer from 2 to 100,000 wherein each pair of adjacent 3D biochip means are operably connected by aligning capillaries present in one of said pair with capillaries present in the other of said pair.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a 4D biochip that overcomes many of the above-noted problems with prior biochip devices and assay systems by providing a means for performing a parallel assay with a large number of biosamples (e.g. patients) for a large number of bioelements in parallel. Such assays are termed herein "parallel-parallel assays". The throughput of a simple experiment using a 4D biochip of the present invention can be higher than the advanced conventional biochips by several orders of magnitude. In addition, the 4D biochip described herein can generate experimental data for multiple biosamples in parallel or at the same experimental time and same experimental conditions. The experimental errors for between biosample comparison are significantly reduced by using the present 4D biochip.

By performing parallel-parallel assays using the present 4D biochips, genotypes can be determined using, for example, traditional gel electrophoresis of PCR products of a target DNA fragment. Taq polymerase used in the PCR reaction is expensive. The present invention 4D biochip can also significantly reduce the amount of reagent use on a per datapoint basis.

The 4D biochip of the present invention can use biosamples directly from microtiter plates with standard formats, e.g., 96-well dishes, if desired, and therefore, no additional liquid handling is needed once the biosamples are processed. Alternatively, the 4D biochip of the present invention can use biosamples delivered by a robotic system conventionally used in traditional biochips, biosamples delivered by hand, or biosamples delivered using any conventional delivery system.

The 4D biochip of the present invention also provides advantageous information flow from biochip design and production to assay and data collection and analyses for relatively large experiments. For example, an experiment involving

1,000 conventional biochips is not unusual. Errors associated with the information flow could potentially result in no or false conclusions. Because of the parallel-parallel nature of the 4D biochip, the information flow can be implemented in a much more simple and systematic way.

The 4D biochip described herein significantly facilitates high throughput screening for drug development and all other genomic and proteomic based research and applications by enabling the processing of large numbers of biosamples (e.g., patients, suspects, antigens, etc.) for a large number of biological factors (e.g., genes, mutations, SNPs, proteins, antibodies, etc.) precisely in a timely and cost effect manner. For example, assaying a single 4D biochip can generate genotypes of 1,536 patients for 1,000 genes using one thousand 1,536-feature 3D biochips to form the 4D biochip. A 4D biochip with the same physical format and different biological format can generate genotypes of 96 patients for 16,000 genes. As another example, assaying using a single 4D biochip can generate interactions among 1,000 antibodies and 1,536 antigens or among 16,000 antibodies and 96 antigens.

The 4D biochip of the present invention provides the ability to obtain high throughput, high efficiency screening of samples. Additionally, the production of the 4D biochip of the present invention provides improvements in both efficiency of production and quality control, relative to conventional single chip methods. In particular, conventional surface-based biochips are produced individually, one at a time. This has the effect of giving a low production yield, since fabrication of 40 glass slides may take up to several days, even using automated robotic based production. A more significant problem with such production is the difficulty in implementing an effective quality control program, since conditions can vary signficantly over those several days. Accordingly, for traditional biochips, the quality

of a single biochip does not represent the quality of the batch of biochips in which it was produced. Thus, the failure rate of conventional biochips when tested for quality control can be significant.

The individual 3D components of the 4D biochips of the present invention, on the other hand, can be fabricated in large quantities, typically in lots of one thousand. These 3D components of the present invention 4D biochips are nearly identical providing significant improvements in quality control and even allowing the use of a portion of the chips for establishment of rigorous quality control measures without an appreciable effect on production efficiency.

Unless defined otherwise, all technical and scientific terms and any acronyms used herein have the same meanings as commonly understood by one of ordinary skill in the art in the field of the invention. Although any methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred methods, devices, and materials are described herein.

All patents and publications mentioned herein are incorporated herein by reference to the extent allowed by law for the purpose of describing and disclosing the proteins, enzymes, vectors, host cells, and methodologies reported therein that might be used with the present invention. However, nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

The 4D biochip of the present invention comprises an assembly of two or more 3D biochips, such as those described in WO 01/26799A1, incorporated herein by reference. In WO 01/26799 A1, a 3D biochip is defined as a channeled capillary array or a bundle of vertical cylinders connected by microchannels on a substrate

Figures 1-3 disclose an embodiment of this 3D biochip for conducting

multiple sample, parallel bioassays, indicated generally by the numeral **10**, which includes the features of the present invention. The biochip **10** generally comprises a plate **20** having an upper surface **30** and a lower surface **40**, a plurality of capillaries **50** defined by the plate **20**, and a plurality of channels **60** selectively and operably connecting adjacent capillaries **50**. Within the context of the present invention, the term "substantially align" is used when referring to capillaries on adjacent biochip plates, and indicates that the capillaries are placed in sufficient alignment to permit passage of sample from one capillary to the other.

In one embodiment, the plate **20** is, for instance, about one centimeter wide by one centimeter long by one-half millimeter thick. Further, the capillaries **50** have, for example, an inner diameter of about 20 microns with about 80 micron spaces between adjacent capillaries **50** such that up to 10,000 capillaries **50** may be fabricated in one biochip **10**. The capillaries **50** are generally disposed about the plate **20** in an array, though many different configurations may also be used. Accordingly, it will be understand that the dimensions and configurations of the plate **20** and the capillaries **50** may vary widely. For instance, the plate **20** according to one embodiment of the invention may be on the order of approximately ten centimeters wide by ten centimeters long by three millimeters thick. In addition, the capillaries **50** may vary, for example, between about 5 microns and about 1000 microns in diameter.

As shown in **Figure 2**, the capillaries **50** further generally increase the effective hybridization surface area of the biochip **10** compared to surface-based biochips using samples comprising a spot on a flat surface. For instance, for a capillary **50** having a diameter equal to the diameter of the spot, the capillary **50** provides a hybridization surface area advantage equal to the ratio of four times the height of the capillary **50** to the diameter of the spot. Thus, for a one-half millimeter

thick plate **20** (500 microns) and a 20 micron capillary diameter, the capillary **50** provides about 100 times the hybridization surface area as compared to a 20 micron diameter spot on a surface-based biochip. A larger hybridization area provides a stronger hybridization signal, increases the hybridization rate, and tends to indicate less false hybridization. In addition, the larger hybridization area tends to reduce the likelihood of secondary structure disturbance since the chance of DNA molecules forming secondary structure is inversely proportional to the hybridization surface area. Thus, for the above example, the channeled capillary array biochip **10** would be 100 times less likely to experience DNA secondary structure disturbance than a surface-based biochip.

Where the biochip **10** includes a high density of capillaries **50**, the plate **20** is comprised of a semiconductor material such as, for example, silicon. A silicon biochip **10** uses, for instance, common semiconductor photolithography and etching processes to form the capillaries **50** in the plate **20**. More particularly, a layer of a photosensitive polymer (not shown) is deposited on a surface of the plate **20** and patterned to define a plurality of holes therein corresponding in the size and distribution to the desired capillaries **50**. The patterned layer thus comprises a mask (not shown) for forming the capillaries **50**. Once the mask is formed, the silicon plate **20** is etched through the holes in the mask to form the plurality of capillaries **50**. Production of a high density biochip **10** is facilitated by using silicon processed by semiconductor fabrication techniques since much greater precision on a smaller scale may thereby be obtained. However, where a low or medium density biochip **10** is required, the biochip **10** may be comprised of a plastic or other polymer material. Where a plastic material is used to form the biochip **10**, a mold is prepared such that the plastic may be, for instance, injection molded to form the plate **20**.

As shown in **Figures 1 and 3**, the capillaries **50** are generally symmetrically and evenly disposed about the plate **20** and extend from the upper surface **30** to the lower surface **40** thereof. Lateral channels **60** are further formed in the plate **20** to selectively operably connect adjacent capillaries **50** about both the upper surface **30** and the lower surface **40**. Note that the orientation-related references used herein, such as "upper," "lower," and "lateral," are used for example only in reference to the corresponding figures. It will be understood that the 3D biochip according to embodiments of the invention may be configured in many different orientations wherein such a reference frame may not be applicable. Preferably, the capillaries **50** are each connected to one adjacent capillary **50** on the upper surface **30** by a channel **60** and to a different capillary **50** on the lower surface **40** by another channel **60**. Most preferably, the channels **60** operably connect adjacent capillaries **50** such that a single continuous passage is formed between at least one reagent inlet capillary **52** and at least one reagent outlet capillary **54**. Thus, for a fluid or reagent probe solution flowing through the passage, the fluid would be directed along the length of the reagent inlet capillary **52**, through a channel **60** on the lower surface **40** to an adjacent capillary **50**, and then along the length of that capillary **50** before being directed through still another channel **60** on the upper surface **30** to another adjacent capillary **50**. This process continues until the fluid is directed serially through all of the capillaries **50** and along the length of the reagent outlet capillary **54**.

According to one embodiment of the present invention, as shown in Figure **3**, the plate **20** may be formed such that the channels **60** operably connecting adjacent capillaries **50** on both the upper surface **30** and the lower surface **40** are open with respect to the upper and lower surfaces **30** and **40**. The open upper surface **30** and lower surface **40** permit access to the capillaries **50** and facilitate immobilization of

the biosample on the inner walls **56** of the capillaries **50**. Once the plate **20** has been prepared with the biosample such that the biosample is adhered to the walls **56** of the capillaries **50**, the upper surface **30** and the lower surface **40** are each sealed, for example, by a glass plate **70**. The glass plates **70** seal the upper surface **30** and the lower surface **40** of the plate **20** such that the ends of the capillaries **50** are sealed and any fluid flowing through the capillary **50** is directed into and out of the capillary **50** by the channels **60**. Accordingly, a fluid, such as the probe solution, is able to flow from the reagent inlet capillary **52** to the reagent outlet capillary **54** along a continuous passage between adjacent capillaries **50**, and serially through each capillary **50** in the plate **20**, to the reagent outlet capillary **54**. Appropriate mechanisms (not shown) are preferably provided in the glass plate **70** sealed to the upper surface **30** (or lower surface **40**) of the plate **20** to allow the flow of a reagent probe solution through the glass plate **70** into the reagent inlet capillary **52** and from the reagent outlet capillary **54**.

According to some embodiments of the present invention, the reagent probe solution flow originates from and returns to a hybridization chamber (not shown) connected to the reagent inlet capillary **52** and the reagent outlet capillary **54**, thereby forming a closed system for a flow of the reagent. Having a closed reagent flow system generally permits a lower quantity of the reagent solution to be used compared to surface-based biochips. For example, a maximum RNA concentration can be attained with 0.1 microliters of a reagent probe solution. In contrast, the Brown chip requires more than 10 microliters of the reagent solution to carry out the hybridization reactions using a surface-based biochip configuration.

High density 3D biochips may be made of a semiconductor material such as, for instance, silicon, which thereby allows semiconductor fabrication techniques to be

applied to form the capillaries and channels as described for example in WO 01/26799 A1.

As noted above, within the context of the present invention, a 4D biochip is defined as an assembly of interconnected 3D biochips as shown in Figure 4, where m 3D Biochips **20** are aligned with each other. The number of 3D biochips (*m*) will vary depending on specific applications with, for example, each of the 3D biochips embedded with one specific bioelement (e.g. probe, peptide, antibody...) for all features (e.g. *n* features). This 4D assembly has potential to assay *n* biological samples (e.g. human, antigen, ...) for *m* bioelements (e.g. genes, proteins, antibodies, ...), wherein *n* is from 1- 100,000, preferably from 10 – 5,000, more preferably from 100-3,000, and *m* is from 1-100,000, preferably from 10-5,000, more preferably from 100-3,000, such that *n* and *m* cannot both be 1 at the same time. The 4D assembly of the present invention can take on a variety of physical shapes and forms, with the most common being a rectilinear solid, preferably in the form of a cube prepared by combining the 3D biochips in a stacked array. Of course, depending on the shape of the 3D biochip, the resulting 4D assembly can have any final shape, so long as the 3D biochips are interconnected as described herein.

The present invention 4D biochip can also be composed with a number *m* of 3D biochips each embedded with *n* different bioelements. The resulting 4D biochip has the potential to assay one biological sample (e.g. a patient) for *n x m* bioelements (e.g. genes or SNPs).

The 4D biochips of the present invention can be used individually, or can be grouped into a larger assembly containing a plurality of 4D biochips. Such a "superassembly" of biochips could be used to further increase the throughput, or to further increase the number of bioelements tested per sample. Such a

"superassembly" could be used in large scale screening operations to screen larger numbers of samples relatively simultaneously. This could be used to replace the conventional expensive laboratory automated screening facilities used by most of the pharmaceutical industry.

For most applications, the design of the 4D biochips will fall in between the two extreme cases, which is further described herein.

An embodiment of the 4D biochip of the present invention with *n* features (cylinders **50**) is depicted in Figure 4. One element, for example, DNA probe, peptide, antibody, etc., is attached to the inside walls of all n cylinders (see WO 01/26799 A1). Thereafter, m biochips, each coated with a unique element can be stacked into an assembly, the assembly therefore containing m elements.

To implement the 4D biochip, two instrument capabilities are needed. One instrument capability is to embed bioelements onto a sufficient number of 3D biochips and the other is to assay the 4D biochip. Of course, the two instrument capabilities can be in separate instruments or combined into a single instrument capable of performing both functions.

Due to the relatively small sizes of the capillaries **50** and the channels **60** and the small amount of the reagent probe solution used during the hybridization process, precise control over the flow rate of the reagent probe solution is desirable. The reagent probe solution can be "pumped" through the capillary **50**. This may be accomplished using electrostatic pressure generated by a voltage applied across the two ends of a capillary **50**. Generally, high voltage of, for example, 1-20kV is required to generate the necessary pressure. By using electrostatic pressure to pump the reagent probe solution through the continuous passage formed by the capillaries **50** and the channels **60**, the voltage may be readily reversed such that the system is

capable of pumping the reagent probe solution in the opposite direction. Alternating directions of the reagent probe solution flow may thereby allow a more complete and faster hybridization process compared to surface-based biochips. For example, several reversals of reagent flow may be necessary to obtain complete hybridization. The movement of molecules by the application of a plurality of electrical fields is further described in U.S. Patent No. 5,126,022 to Soane *et al.*, the contents of which are herein incorporated by reference.

Of course, it may be desirable to arrange the various 3D biochips in a particular arrangement such that as the sample passes through the individual capillary in one 3D biochip into the capillary in the adjacent 3D biochip, the possibility of sequential interactions (selective hybridizations, selective removal of sample components) can occur. In such a case, the sample would be most desired to pass through the 4D biochip in a single direction, instead of the above-described alternating flow directions.

When preparing the 4D biochip of the present invention, the 3D biochips are attached to one another, in a stacked array as described above, using any of a variety of methods of maintaining plate to plate contact while maintaining alignment between capillaries on adjacent plate faces. This can be accomplished by using mechanical guides for the 3D biochip plates to hold them in place, or by using an adhesive or glue means. Of course, if adhesive or glue means is used, it is necessary to carefully apply the adhesive or glue to avoid plugging one or more capillaries. The adhesive or glue means can be any conventional adhesive or glue that is compatible with the material of the 3D biochip and will maintain the bond between adjacent 3D bioehips in the assembly. The mechanical guides that can be used in one embodiment of the present invention can include external guides that align the 3D biochips with one another, or

can be internal guides or pins which pass through the stacked array of 3D biochips. Alternatively, both external and internal guides can be used, such as a combination of a plurality of pins, preferably 3-5 pins arranged through the individual plate, along with a shell or box around the outer perimeter of the assembled 4D biochip. The outer shell or box can be made of any desired material, preferably of metal, plastic or glass, so long as it does not interact or interfere with the samples or bioelements contained in the 4D biochip assemble. As an alternative embodiment, the 3D biochips can be prepared such that one side contains indentations, while the other side contains protrusions, such that upon stacking two or more 3D biochips, the protrusions from one 3D biochip fit into the indentations of the adjacent 3D biochip.

According to chemical kinetics, the total number of collisions between the DNA fragments in the biosample and the complementary strand in the probe solution is a function of the hybridization area, the concentration of the complementary strand in the probe solution, and the velocity of the DNA molecules in the probe solution. Thus, control of the flow rate of the probe solution is advantageous compared to the random flow used by surface-based biochips. The use of small diameter capillaries **50** with controlled flow of the probe solution further increases effective concentration of the complementary RNA strands. In one embodiment, for example, the effective concentration of the RNA strands in the probe solution may be up to 300,000 times greater than a comparable surface-based biochip. Thus, control of the probe solution flow and the higher effective hybridization area provides more complete hybridization, with a higher hybridization rate, and reduces the amount of the probe solution required to perform the assay. In one embodiment, for example, the time necessary to complete a hybridization procedure may be on the order of minutes as

compared to hours of hybridization time that may be required for surface-based biochips.

In another embodiment, a volume reducing liquid assayer, as described in WO 01/26798 A1, which is incorporated herein by reference, can be employed to deliver the biosamples to the 4D biochip having n features and m biochips. By controlling fluids between the delivery device and the 4D biochip device, the m elements are assayed for n biological samples. This can facilitate high throughput screening for a number of samples. Each 4D biochip will generate n x m data points, e.g., n x m = 1536 x 1000= 1,536,000 data points. In one embodiment, the 4D biochip with or without the delivery device described above, can be useful for efficiently screening clinical diagnostic genes or markers (m genes or markers) for n patients.

The volume-reducing liquid arrayer is depicted in Figures 5, 6, 7A,7B, 8A, and 8B. Figures 5 and 6 disclose embodiments of a volume-reducing arrayer apparatus adapted to deliver a predetermined amount of a liquid solution to a substrate, the arrayer apparatus (also referred to herein as an "arrayer") being indicated generally by the numeral **110** and including the features of the present invention. The arrayer **110** generally comprises a reservoir **120**, at least one arrayer block **140**, a flow control device **160**, and a stamper head **180** cooperating to deliver biosamples in the form of a liquid to a biochip **200**.

In one embodiment of the arrayer, the reservoir **120** comprises, for example, a microtiter plate constructed so as to store bulk DNA solutions therein. More particularly, for example, a 20cm x 20cm microtiter plate may contain a plurality of wells **122** arranged in an array, where each well **122** is capable of holding 5 to 200 microliters of a DNA solution. Thus, according to current technology, such a microtiter plate may, for example, hold as many as 10,000 DNA samples therein and

may theoretically be used to fabricate approximately 5,000 to 10,000 separate biochips **200**.

Underlying the reservoir **120**, according to one advantageous embodiment, is at least one arrayer block **140**, in the form of a plate, defining at least one and, in some instances, a plurality of arrayer capillaries **142**. The arrayer capillaries **142** may be arranged in an array corresponding to the array of wells **122** in the reservoir **120**. The wells **122** in the reservoir **120** may each have a valve (not shown) for controlling the flow of the liquid solution, and thus the biosamples, from the wells **122** into the corresponding arrayer capillaries **142** in the arrayer block **140**. It is understood, however, that delivery of the solution from the wells **122** to the arrayer capillaries **142** may be accomplished by other delivery methods, wherein the reservoir **120** is in communication with, but not necessarily disposed atop, the arrayer block **140**, consistent with the spirit and scope of the present invention.

A typical biochip **200** has a surface area on the order of, for example, about one square centimeter to about four square centimeters and requires microscopically-sized samples to form the necessary oligonucleotide probes on the biochip **200**. The typical biochip **200**, although shown as a square shaped device, can optionally take on any desired shape, including but not limited to, circular, oval, polygonal, etc. The liquid solution (also referred to herein as the "biosamples') released from the wells **122** must be reduced with respect to both the volume of the individual biosample as well as the area occupied by the well array **122**.

The arrayer block **140** is thus configured to receive and reduce the volume of the biosamples from the reservoir **120** and to reduce the area occupied by the well array **122**. As shown in Figures 7A and 7B, the arrayer block **140** is typically a flat plate having an upper surface **144** and a lower surface **145** and is sealed and adhered

to the reservoir **120** by a thin film of silicon. The arrayer capillaries **142** defined by the arrayer block **140** are arranged in an array corresponding to the well array **122** in the reservoir **120** and each arrayer capillary **142** may extend, in some instances, from the upper surface **144** to the lower surface **145** of the arrayer block **140**. At the lower surface **144** of the arrayer block **140**, an array of indentations **146** is formed, wherein the indentation array **146** corresponds to at least a portion of the arrayer capillary array **142**. In order to reduce the area occupied by the arrayer capillary array **142**, the indentation array **146** is typically formed, for instance, toward the center of the arrayer block **140** and inwardly of at least some of the arrayer capillaries **142**, though a variety of different configuration may be used in accordance with the spirit and scope of the present invention. A plurality of channels **148** are also formed in the lower surface **145** of the arrayer block **140**, with each channel **148** extending between an arrayer capillary **142** and a corresponding indentation in the indentation array **146**. As shown in **FIGS. 7A, 7B, 8A** and **8B**, the reduction in the arrayer capillary array **142** area may be accomplished with a single arrayer block **140**, wherein the entire arrayer capillary array **142** is reduced in area to the size of the indentation array **146** by the single arrayer block **140**.

As shown in Figures 8A and 8B, reducing the arrayer capillary array **142** area may be alternatively accomplished with multiple arrayer blocks, wherein a first arrayer block **140** and a second arrayer block **150** are shown in this embodiment, and wherein each successive arrayer block reduces the area occupied by a particular subset of the first arrayer capillary array **142**. For example, the second arrayer block **150** may be disposed below the first arrayer block **140** such that the lower surface **145** of the first arrayer block **140** is sealed by the upper surface **154** of the second arrayer block **150**. The upper surface **154** of the second arrayer block **150** seals the first

arrayer capillaries **142** in the first arrayer block **140** having channels **148** connected thereto, such that each first arrayer capillary **142** having a connected channel **148**, and that corresponding channel **148**, are sealed. Accordingly, any portion of the solution flowing through the first arrayer capillary **142** flows through the channel **148** to the corresponding indentation **146**.

The second arrayer block **150** further defines a plurality of second arrayer capillaries **151** therein corresponding to the array of indentations **146** in the first arrayer block **140**. The second arrayer capillaries **151** typically extend through the second arrayer block **150** from the upper surface **154** to the lower surface **155** thereof. In order to reduce the volume of the biosample solution in each capillary, the second arrayer capillaries **151** in the second arrayer block **150** are configured to have a smaller diameter than the first arrayer capillaries **142** in the first arrayer block **140**. For example, in one embodiment of the present invention, the first arrayer capillaries **142** of the first arrayer block **140** have a diameter of between about one millimeter and about two millimeters while the second arrayer capillaries **151** in the second arrayer block **150** have a diameter of between about 50 microns and about 200 microns. The smaller diameter of the second arrayer capillaries **151** in the second arrayer block **150** reduces the volume per unit length of the solution in the capillary and provides more accurate and easier regulated volumetric control over the flow of the biosample solution from the reservoir **120** to the biochip **200**. Thus, it will be understood that, by reducing the dimensions and the spacing of subsequent sets of capillaries, the volume of the individual biosample as well as the area occupied by the corresponding capillary array are accordingly reduced in order to scale the biosamples to the dimensions of the corresponding biochip according to the spirit and scope of the present invention.

In some instances, the first arrayer block **140** may have first arrayer capillaries **142** which are not connected to channels **148** at the lower surface **145** of the first arrayer block **140**. In those instances, the second plate **150** also defines a plurality of continuation capillaries **152** corresponding to the unchanneled first arrayer capillaries **142** of the first arrayer block **140** and being configured to have a substantially similar diameter with respect thereto. Accordingly, when the first arrayer block **140** is engaged with the second arrayer block **150**, some of the first arrayer capillaries **142** continue through the first arrayer block **140** to the corresponding continuation capillaries **152** in the second arrayer block **150**. Where the second arrayer block **150** continues some of the first arrayer capillaries **142** of the first arrayer block **140** with continuation capillaries **152**, the second arrayer block **150** also includes channels **158** defined in the lower surface **155** thereof, with the channels **158** leading to an array of corresponding indentations **156** also defined by the lower surface **155** of the second arrayer block **150**. In such instances, the second arrayer block **150** completes the reduction of the area of the original capillary array **142**. It is understood, however, that the reduction in the area of the capillary array may be accomplished by various methods consistent with the spirit and scope of the present invention. The various methods of reducing the area of the capillary array will be applicable herein with respect to the described embodiments in addition to or in the alternative to the specific configuration described.

Once the capillary array **142** has been reduced to the desired area and the individual biosamples have been reduced to the desired volume, a flow control **160** is operably connected to the lower surface **155** of the last arrayer block **150**. The flow control device **160** may include, for example, an array of flow control capillaries **162** corresponding to the previous reduced area capillary array formed by the arrayer

blocks. The flow control device **160** is configured to control the flow of the biosamples through the flow control capillaries **162** and may take the form of, for instance, a valve, a pump, or the like.

The movement of molecules by the application of a plurality of electrical fields is further described in U.S. Patent No. 5,126,022 to Soane et al., the contents of which are herein incorporated by reference. However, flow control of the biosamples as mentioned herein may also be accomplished, for instance, with an external pump interfaced with the arrayer apparatus **110** for pumping the biosamples through the capillaries, instead of using an electrostatic pump configured as previously described. Alternatively, for example, an external valve may be engaged with the arrayer apparatus **110**, for controlling the flow of the biosamples. Thus, it will be understood that flow control of the biosamples may be achieved in many different manners, in addition to that described, according to spirit and scope of the present invention.

Another advantageous aspect of a volume-reducing arrayer apparatus according to the present invention comprises a method of fabricating a volume-reducing arrayer apparatus adapted to deliver a predetermined amount of a liquid solution from a reservoir to a substrate as described in WO 01/26798.

Bioelements (e.g. DNA probes) in the reservoirs **120** are deposited into the cylinders of biochip **200** through channels connecting the reservoirs **120** to the corresponding cylinders on the biochip **200**. If a 4D biochip of the present invention (see Figures 4 and 9) composed of m 3D biochips replaces the single 3D biochip **200**, then a single operation (or stampings) will deposit all *n* bioelements onto all *m* 3D biochips.

By taking one 3D biochip from each of the *m* stampings, a 4D biochip is assembled with *m* 3D biochips. Biochips from the *m* stampings will assemble *m* 4D

biochips. The arrayer can be used for assaying the 4D biochip by placing the cube in the place of biochip **200** and filling the reservoirs **120** with biosamples.

For reducing liquid handling and simplifying the biostamper instrument, the plate **110** (Figures 5-8B) can be replaced with a standard micro titer plate (96-well or 384-well or 1536-well) and the connecting channels **48** (Figures 7A/B and 8A/B) can be replaced with standard tubing (e.g. peek tubing) (Figure 10). The reservoir **120** is now replaced with well of the microtiter plate. Instruments from the tubing approach can be used for both embedding the 3D biochips and assaying the 4D biochip. Bioelements for coating the biochips or biosamples for assaying the biochips can be placed in the wells of the microtiter plate (or plates). By connecting to a syringe pump to the top of the 4D biochip, bioelements or biosamples (in liquid form) can be drawn into the cylinders of the biochips for coating or assaying. (Figure 11) whereby the 4D biochip is held in place by a biochip holder, which holder is placed directly above a microtiter plate having n wells containing biological probes or samples as accordingly required. The biochip holder can hold a large number of biochips, e.g., up to 100,000, each coated with different probes, antibodies or antigens. Upon drawing the fluids upwards with, for example, a syringe, the samples or probes will be drawn into the *m* biochips in the biochip holder. This delivery method can be used to coat the biochips with probes or can be used to deliver the samples to be assayed. Alternative methods of providing a mechanism for moving reagent solutions may include vacuum or electric charge as described herein.

To prevent contamination due to potential overflow, tubing extension on the top the biochip holder can be installed (Figure 12). Overflow tubings are pressure fitted in the top tier of the 3D biochip holder. Reagent flow in each of the features can visually check or by automatic image analysis using a CCD camera. The

overflow of the reagents (or probes, human samples, or others) will not contaminate each other.

To implement the 4D biochips, two major steps are involved. Step 1 is to stamp a sufficient number of 3D biochips with target bioelements (e.g. DNA probes) and assemble them into the 4D biochip. The other step is to assay the assembled 4D biochips using biosamples containing the experimental targets (e.g. mutations, SNPs, genes, proteins, DNA transcripts or RNAs, etc.). For a given experiment with certain numbers of targets (e.g. genes) and biosamples (e.g. patients) and formats of the 3D biochips (e.g. number of features and the correspondence between the features and the standard format of the microtiter plates), there is an optimum design for implementing the experiment using the 4D biochip.

An example of preparing a 4D biochip design for 384-feature biochips having a format corresponding to the format of 4 standard 96-well microtiter plates is provided below. The procedures described can be readily adapted by those of ordinary skill to use for other arrangements.

For an arrangement of a 384-feature 3D Biochip for a parallel-parallel assay in a 4D Biochip, the biochip can preferably be composed of 4 regions, preferably equal in size, each having 96 capillary channels thereby having 96 features per quadrant. In such a 384-feature biochip, there are numerous arrangements for coating and assaying the biochips. For example, 4 different probes coated on the chip with 96 replications. The 96 replications more preferably correspond to the geometry of a 96-well microtiter plate. This arrangement will allow assaying 96 bio-samples in parallel. If a 4D Biochip with 1,000 3D biochips (having 384-features as described) is employed, then the 4D biochip has the potential for assaying 96 biosamples for 4 x1000 = 4,000

genes or other types of elements thereby generating 96 x4 x1,000=384,000 data points.

The arrangement of a 4D biochip can be very flexible to meet the needs of certain applications. In some applications, extreme distributions of samples or probes can be used, for example, where all 384 features are different or where all 384 features are the same. Where all 384 features are different, a 4D biochip made of 1000 3D biochips has the potential of assaying 1 biosample for 384,000 genes or other types of elements, which could be of particular practical use for screening an individual for various disease related genes. Where all 384 features are the same, a 4D biochip made from 1000 3D biochips can be used to assay 384 biosamples for 1,000 genes and generating 384 x 1000=384,000 data points.

An arrangement for assaying, for example, whole human genome samples, using a 4D biochip of the present invention is illustrated below. In this illustration a 4D Biochip arranged in the following combination (with a 384-feature 3D Biochip as an example) would require the following number of 3D biochips:

Number of 3D biochips for 40,000 genes	Number of bio-elements on a 3D biochip (e.g., probes, etc.)	Number of bio-samples for a parallel assay
40,000	1	384
20,000	2	192
10,000	4	96
5,000	8	48
3,333	12	32
2,500	16	24
1,667	24	16
1,250	32	12
833	48	8
417	96	4
208	384	1

As a further illustration, in 4D Biochips comprising 250, 500, or 1000 3D Biochips, the following combinations can be illustrated: Five 250-3D cubes have a potential to assay 40,000 genes for 12 human samples; Five 500-3D cubes have a potential to assay 40,000 genes for 24 human samples; Five 1,000-3D cubes have a potential of assaying 40,000 genes for 48 human samples; etcetera. Potentially, there is no limit to the arrangement of 4D biochips for either large or small applications.

To genotype 6,000 human samples for 20,000 genes using a 384-feature 3D-biochip to form a 4-D biochip various combinations are possible. For example, to generate 6,000 x 20,000 = 120,000,000 data points, approximately 312,500 384-feature 3D biochips are needed. If 384-feature 3D biochips form a 4D biochip, 500 4D biochips would be required for the experiment. To obtain the 500 4D biochips,

625 stampings are needed to produce 500 3D biochips for each of the stamping processes. The arrangement of a 3D biochip is shown in Figure XX, where each 3D biochip carries 32 unique genes with 12 replications. The 12 replications are for assaying 12 human individuals. The 625 stampings will produce 625 types of 3D biochips, which collectively carry the 20,000 genes. Then the 500 x 625 3D biochips will generate 500 4D biochips of 625 3D biochips each.

Each of the 500 4D biochips contains 20,000 genes and for genotyping 12 individuals. The 500 4D biochips can genotype 6,000 individuals for all 20,000 genes. For different applications, there are different arrangements to optimize the power and cost. For example, to assay 2,000 genes and 600 human samples, each 3D biochip has 32 genes for 12 human samples. In 63 stampings, 2016 genes are coated-fifty cubes with 63 3D biochips will genotype the 600 human samples for the 2,000 genes.

The 4D biochip of the present invention is also useful for very efficiently genotyping relatively small numbers of genes for a large number of human samples. For example, using the present invention 4D biochips, one can genotype 500 genes for 20,000 human samples using 52 4D biochips.

The following illustrates a method of determining the arrangements of the 4D-biochip design needed for a particular example (however, it is to be understood that this is not the only method for determining such arrangements, but merely exemplary of a variety of methods that would be readily determined by one of ordinary skill upon reading the present description):

Definitions:

G = number of total targets (e.g., genes)

P = number of samples (e.g., patients)

GP= number of data points

N= number of features in a 3D biochip

GP/N= number of 3D biochips needed

g = number of genes in a 3D biochip

G/g = X = number of 3D biochips in a 4D cube = also number of stampings

N/g = number of samples for each cube

P/N/g = Pg/N = Y = number of 4D cubes

X = G/g; Y= Pg/N;

We want $X + Y = \frac{G}{g} + \frac{Pg}{N} = \frac{NG + Pg^2}{NG} \rightarrow$ minimum (the smallest combined number of stampings and assays) or $\frac{Ng}{NG + Pg^2} \rightarrow$ maximum.

Set $\left[\frac{Ng}{NG + Pg^2}\right]' = 0$ (Note: set the first derivative of $\frac{Ng}{NG + Pg^2}$ with respect to g to be 0)

We have $NG - Pg^2 = 0$ and the solutions are

$g = \sqrt{\frac{NG}{P}}$

$X = Y = \sqrt{\frac{PG}{N}}$

Using the formulas disclosed above, the following examples illustrate how these formulas are employed:

Where N = 384 features; G = 2,000 genes; P = 5,000 patients:

$$g = \sqrt{\frac{384 \times 2000}{5000}} = 12$$

X = 2000/12 = 161 stampings

Y = 5000 x 12.39/ 384 = 161 cubes

Where N = 384; G = 200; P = 500:

$$X = Y = \sqrt{\frac{200 \times 500}{384}} = 16$$

In these two examples, demonstrate a primary advantage of the 4D biochip of the present invention. To genotype 5,000 patients for 2,000 genes, only 161 + 161 = 322 operations are required. To genotype 500 patients for 200 genes only 16 + 16 = 32 operations are required. To genotype all 35,000 genes for 5,000 patients, only 675 + 675 =1350 operations (675 stampings and 675 assays) using a 384-feature biochip would be required. However, if a 1536-feature biochip is used for the task, only 337 =337 = 675 operations are needed.

The user may desire to configure or arrange a 4D biochip depending on monetary and/or time considerations. Therefore, X and Y can be weighted by costs or time, dollars, duration, etc. For example, if "a" is a weight for X and "b" is a weight for Y, then to minimize the following is illustrated:

$aX + bY \rightarrow$ minimum

$aX = b\ Y = aG/g + bPg/N = (aNG + bPg^2)/ Ng$

Set: $\left[\frac{Ng}{aNG+bPg^2}\right]' = 0$ (note: set the first derivative of $\frac{Ng}{aNG+bPg^2}$ with respect to g to be 0)

:

We have the solutions:

$$g = \sqrt{\frac{aNG}{bP}}$$

$$X = \sqrt{\frac{bPG}{aN}}$$

$$Y = \sqrt{\frac{aPG}{bN}}$$

X/Y = b/a

Therefore, if N = 384; G =2,000; P= 5000; a=1; b=2:

X = 228.2 ≈ 229

Y = 114.1 ≈ 115

An application of employing the 4D biochip of the present invention is illustrated below:

To relate variations in gene expression to the molecular pharmacology of cancer and link bioinfomatics to chemoinformatics Scherf et al (Scherf et al, (2000) A gene expression database for the molecular pharmacology of cancer. *Nature Genetics*: 24:236-244.) generated relationships between drug activities and gene expression levels that are correlative, not causal, and they generate a hypothesis that must be tested. In this study the Cell line x gene (60 x 1,376) used 60 DNA microarrays (biochips) and cell line x compound (60 x 118). However, problems with the experiments described in this study are that the two data matrices generated are

disconnected data sets in terms of gene expression responding to compound treatment. The results cannot be used directly for drug development. Furthermore, the relationship between drug activities and gene expression were based on correlation coefficients between the two disconnected data sets; and no direct connection between gene and compound treatments and no direct drug targets were identified.

Three-D data of cell line x gene x compound will result in a direct relationship between gene and compound treatments (drug targets and lead compounds). However, 7080 traditional biochips are needed to implement the experiment. The experiment is not feasible using conventional technology because fabricating and assaying the 7080 biochips would cost a significant (and prohibitive) amount of money and time. Most importantly, even the experiment can be implemented using the current DNA microarray technology the data may not result in any meaningful biological conclusion because of experimental errors and mishandling of the information flow.

Using the 4D biochip in this invention, this experiment can be implemented cost effectively, timely and accurately. To implement this experiment, 172 stampings and assaying 172 4D biochips, a total of 344 operations, are needed. The 4D biochip size will be 172 3D biochips. A total of 172 4D biochips can potentially generate 172 x 172 x 384 = 11,360,256 data points which will cover the needed 9,742,080 data points for the experiment and a large number of replications for quality control and statistical analysis.

Obviously, numerous modifications and variations on the present invention are possible in light of the above teachings. It is therefore to be understood that within

the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.